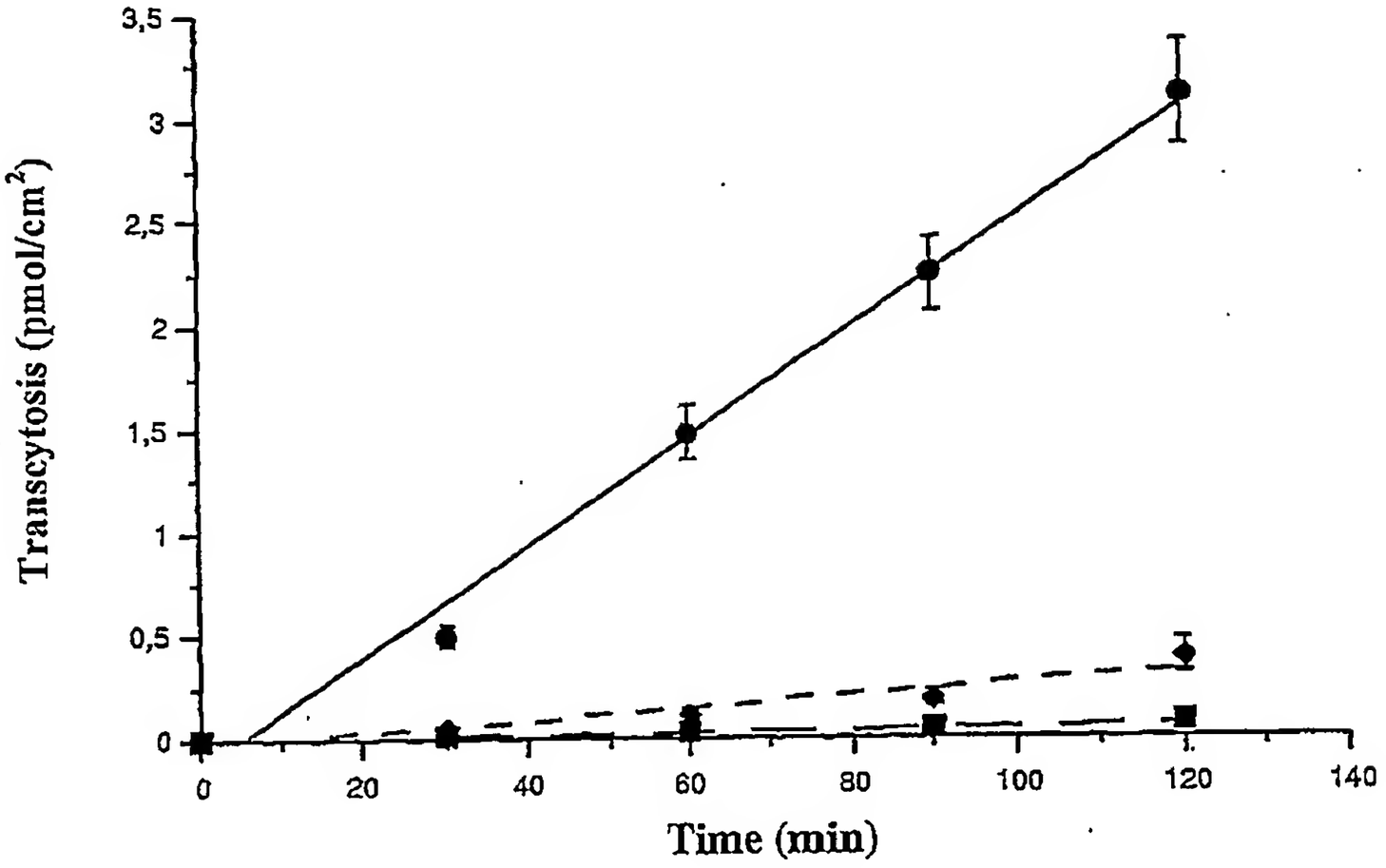

Transcytosis (pmol/cm²)
3,5
3
2,5
2
1,5
1
0,5
0
0
20
40
60
80
100
120
140
Time (min)

FIG. 1

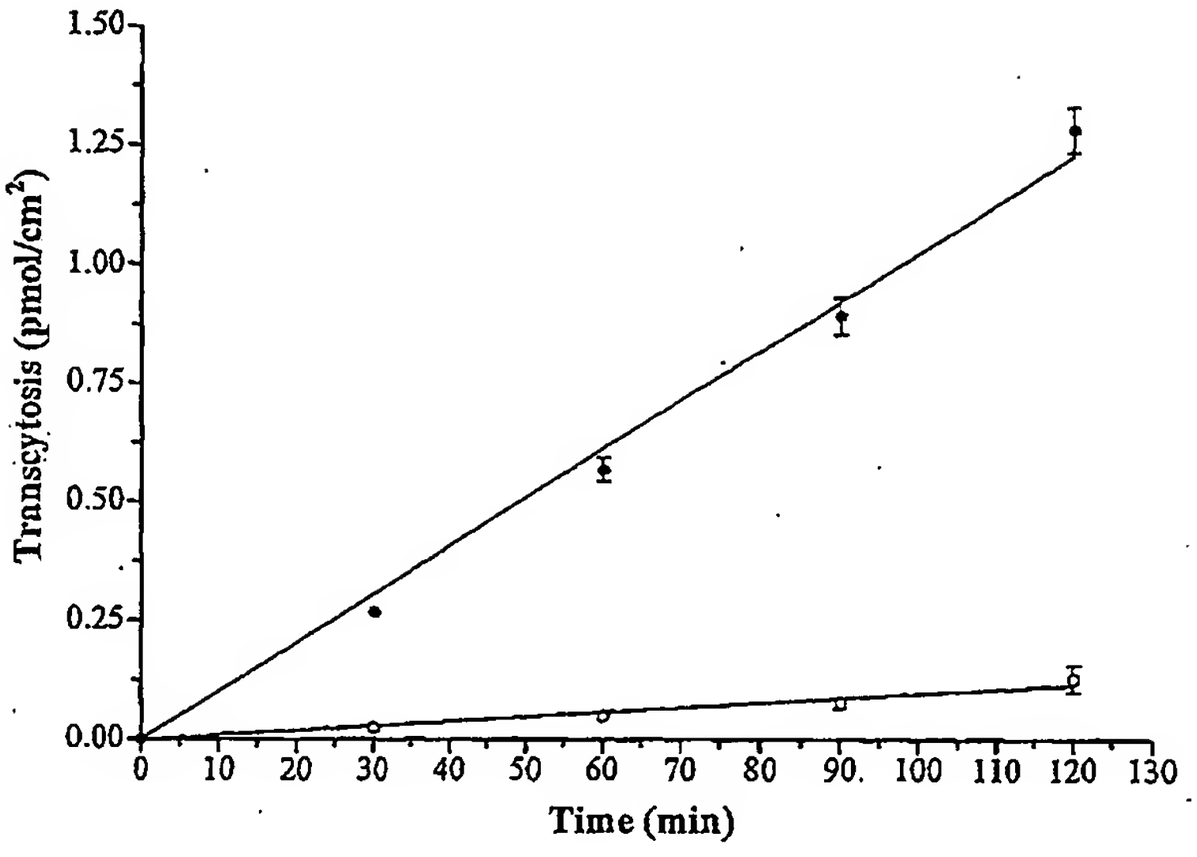

Transcytosis (pmol/cm²)
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0
10
20
30
40
50
60
70
80
90.
100
110
120
130
Time (min)

FIG. 2

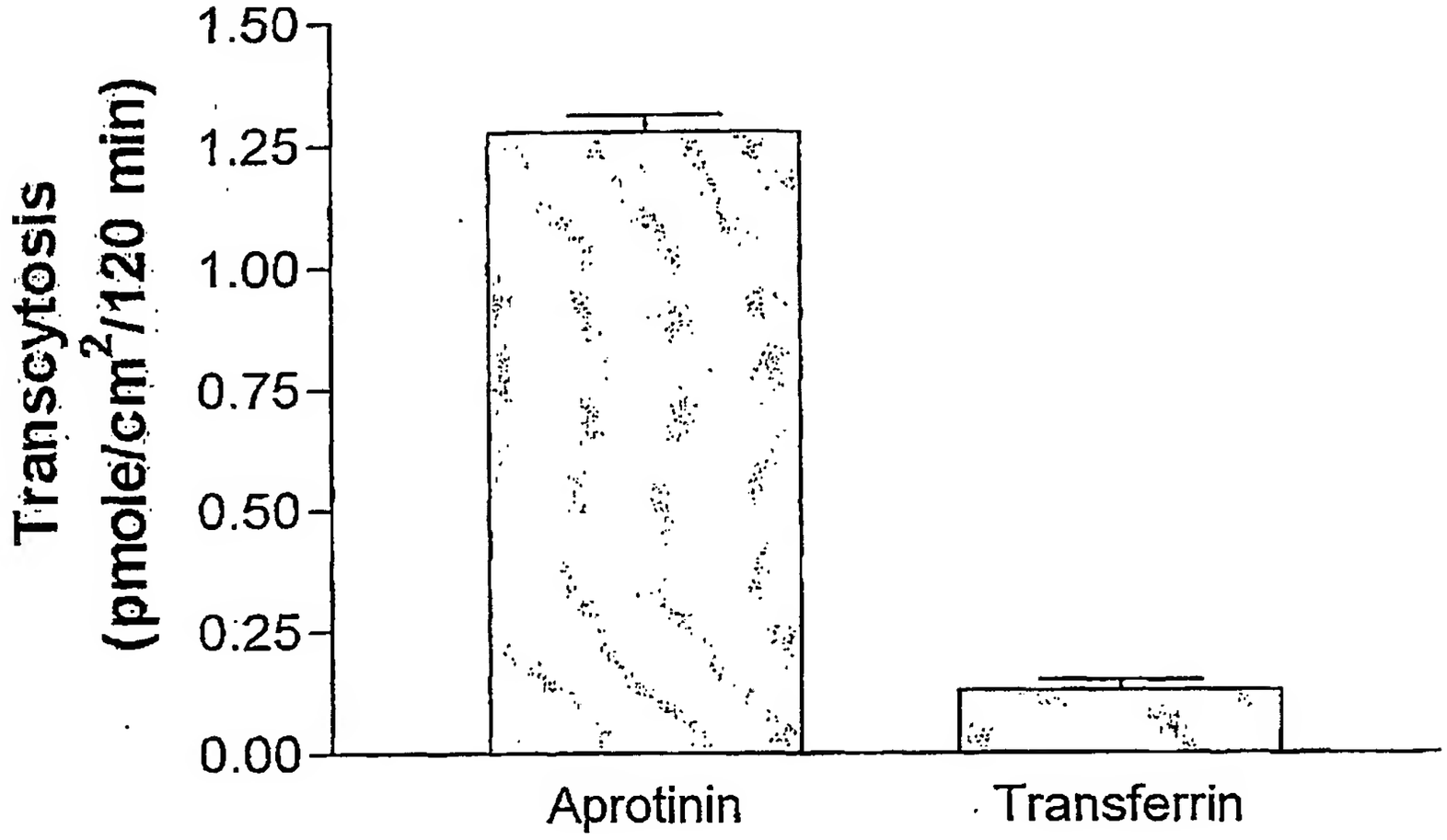

Transcytosis
(pmole/cm2/120 min)
1.50
1.25
1.00
0.75
0.50
0.25
0.00
Aprotinin
Transferrin

FIG. 3

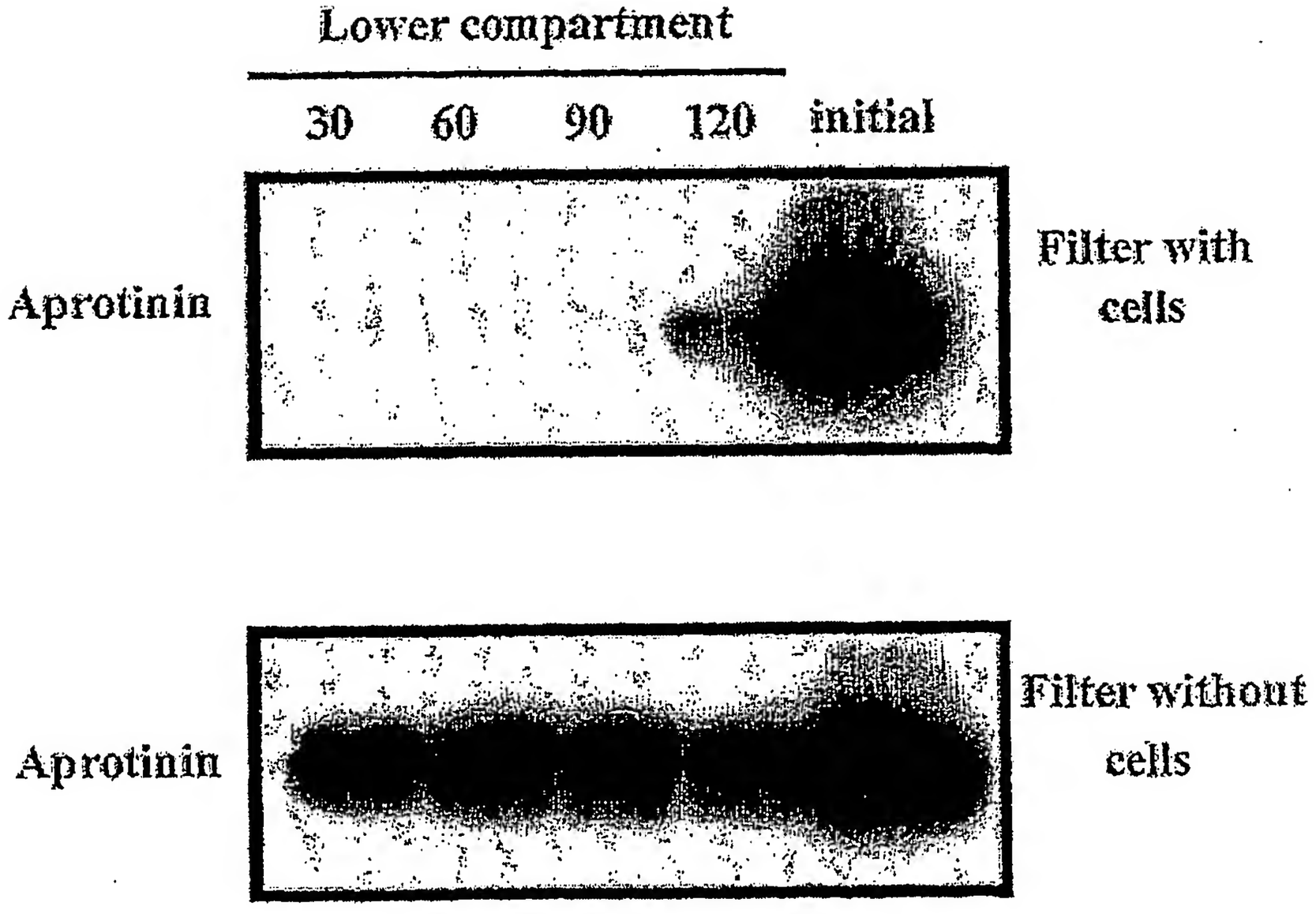

Lower compartment
30
60
90
120
initial
Aprotinin
Filter with cells
Aprotinin
Filter without cells

FIG. 4

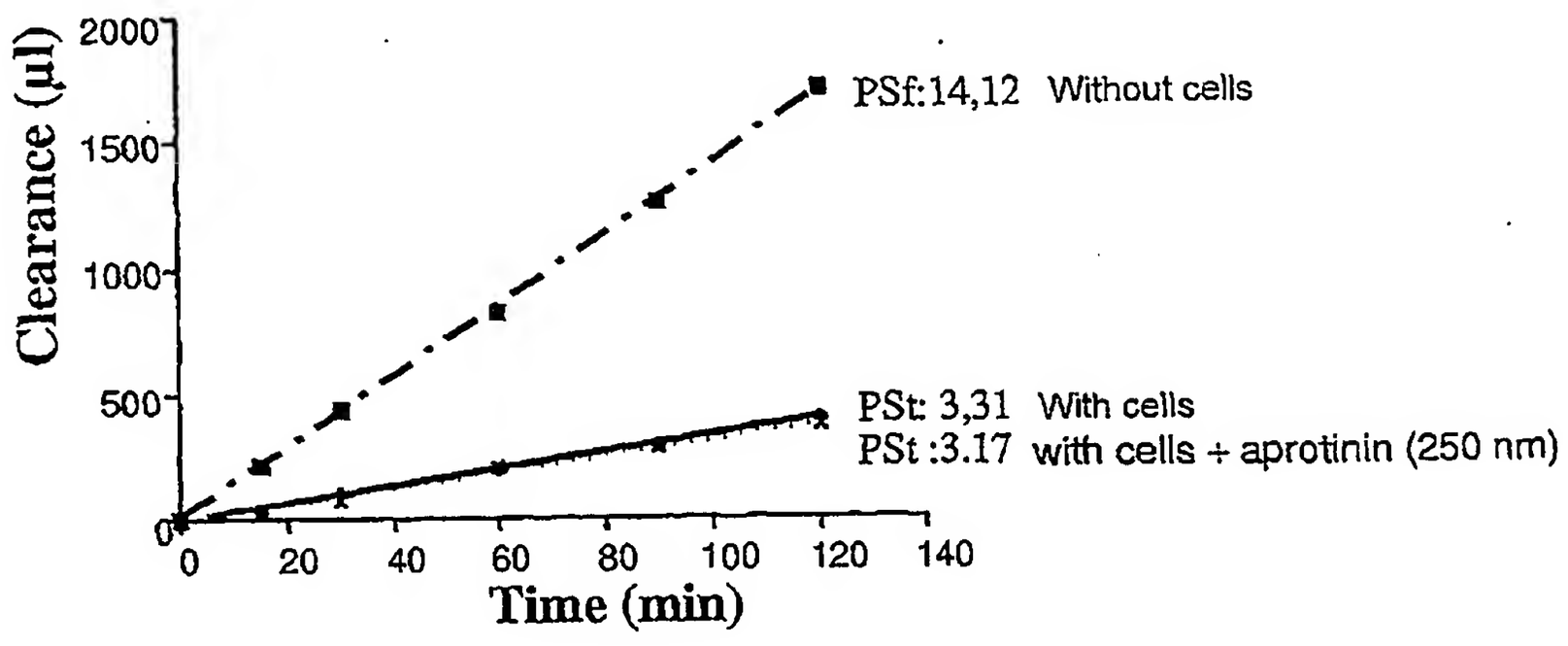
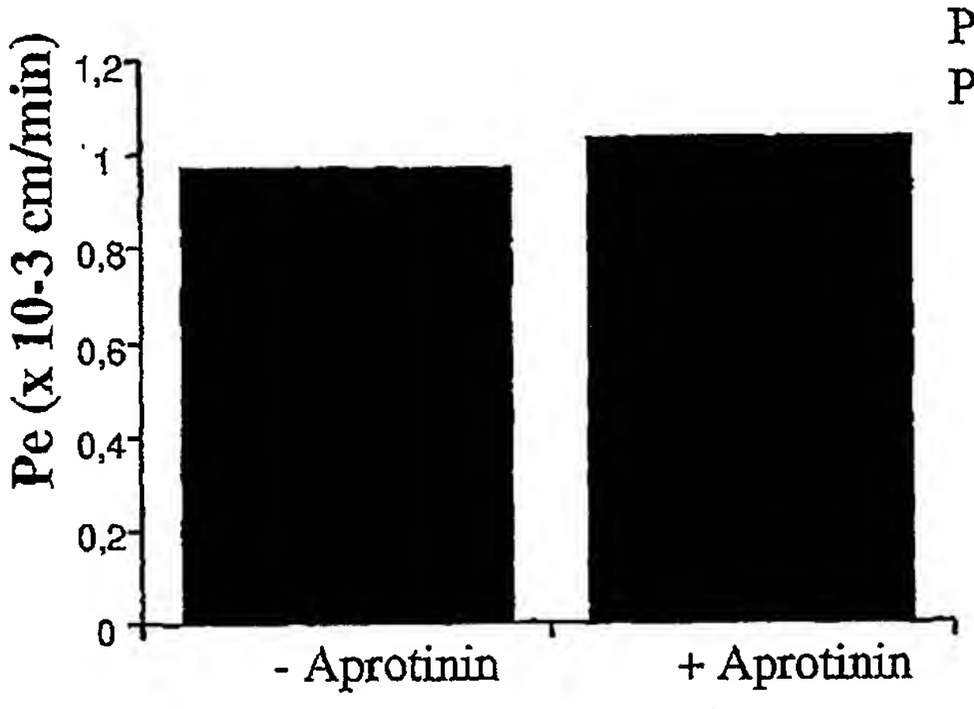

Clearance (µl)
2000
1500
1000
500
0
0
20
40
60
80
100
120
140
Time (min)
PSf:14,12 Without cells
PSt: 3,31 With cells
PSt :3.17 with cells + aprotinin (250 nm)

FIG. 5



Pe without aprotinin: 0.97
Pe with aprotinin: 1.03

FIG. 6

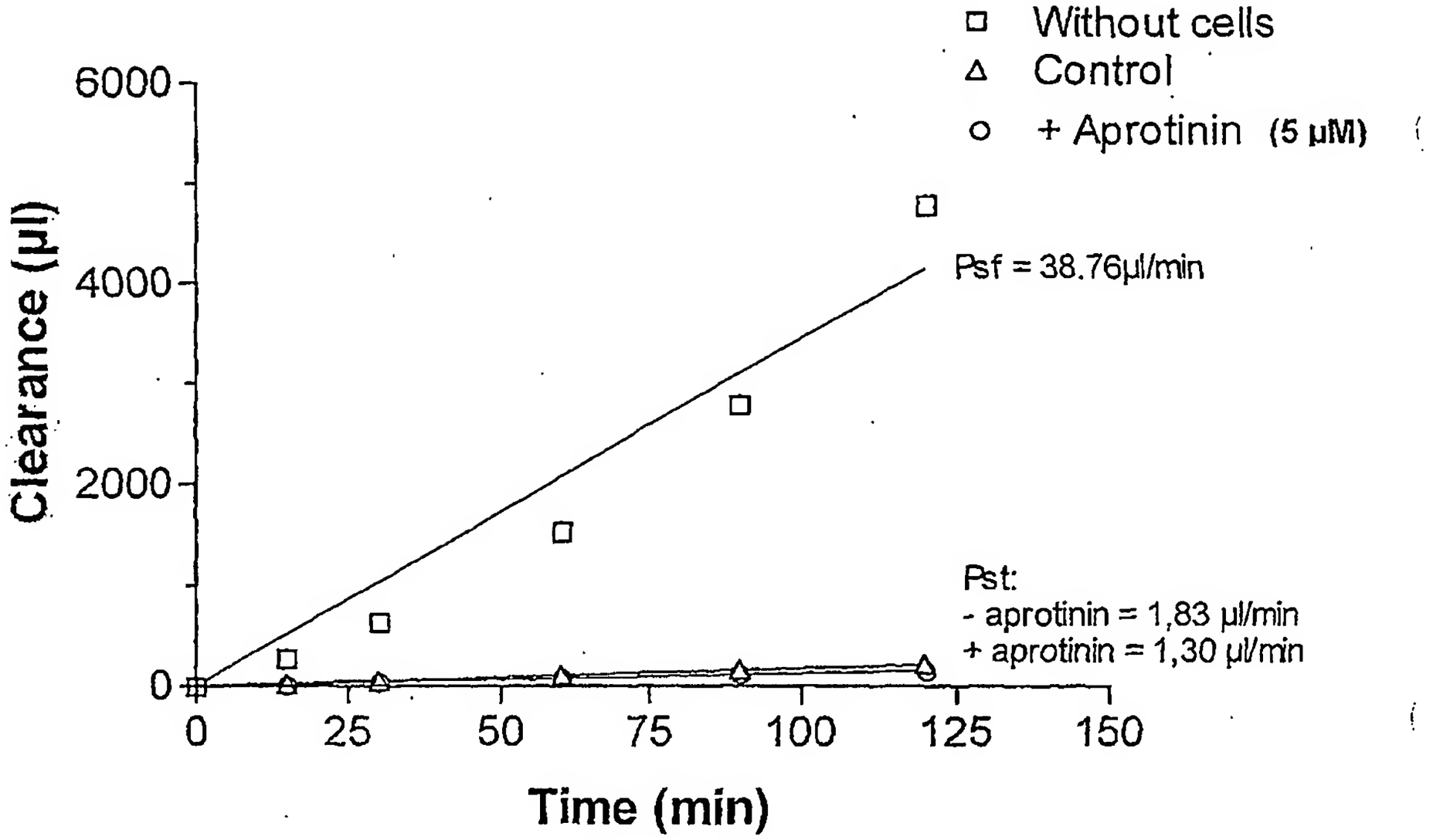

Without cells
Control
+ Aprotinin (5 µM)
Psf = 38.76µl/min
Pst:
- aprotinin = 1,83 µl/min
+ aprotinin = 1,30 µl/min
Clearance (µl)
6000
4000
2000
0
0
25
50
75
100
125
150
Time (min)

FIG. 7

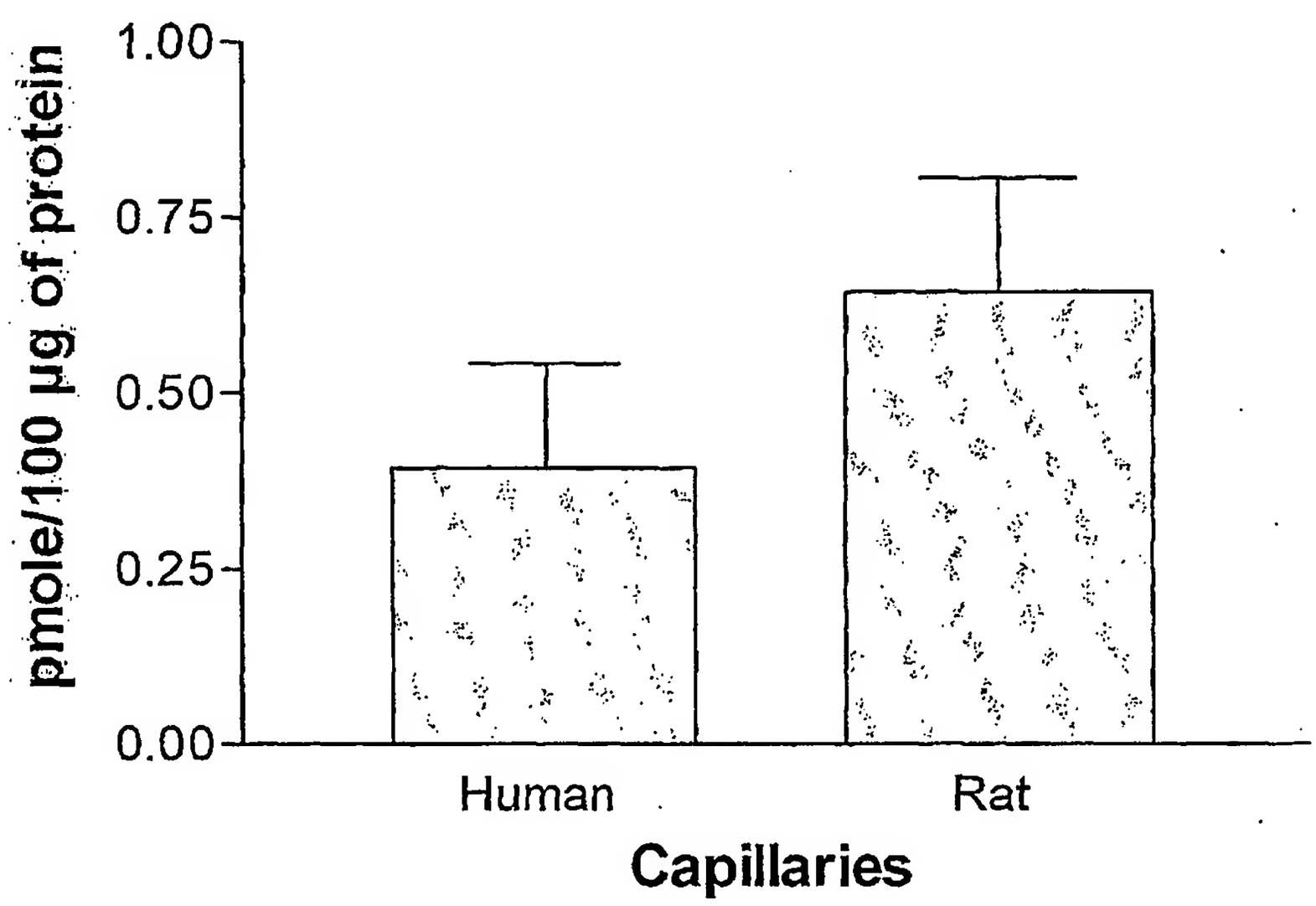

pmole/100 µg of protein
1.00
0.75
0.50
0.25
0.00
Human
Rat
Capillaries

FIG. 8

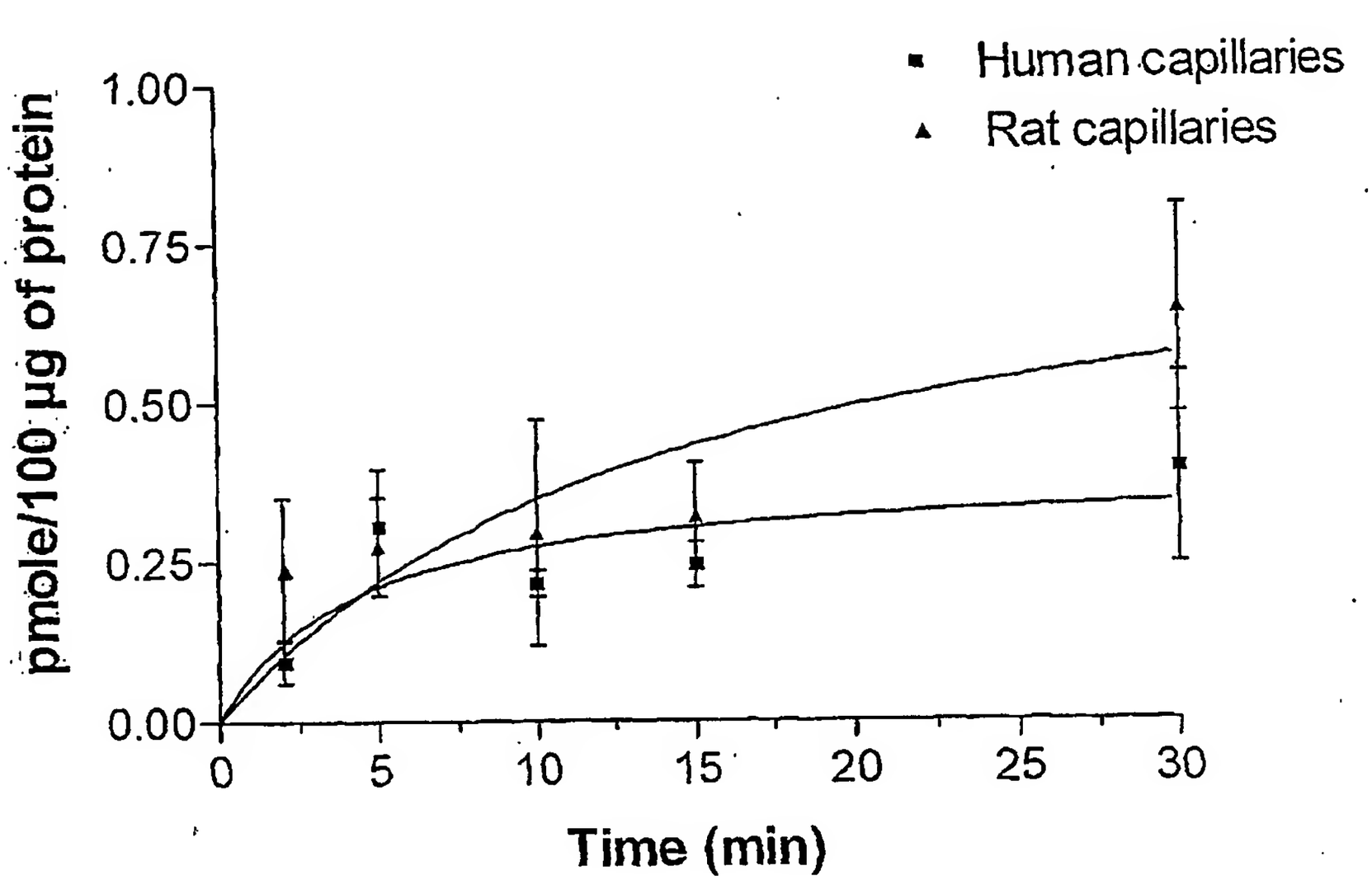

Human capillaries
Rat capillaries
pmole/100 µg of protein
1.00
0.75
0.50
0.25
0.00
0
5
10
15
20
25
30
Time (min)

FIG. 9

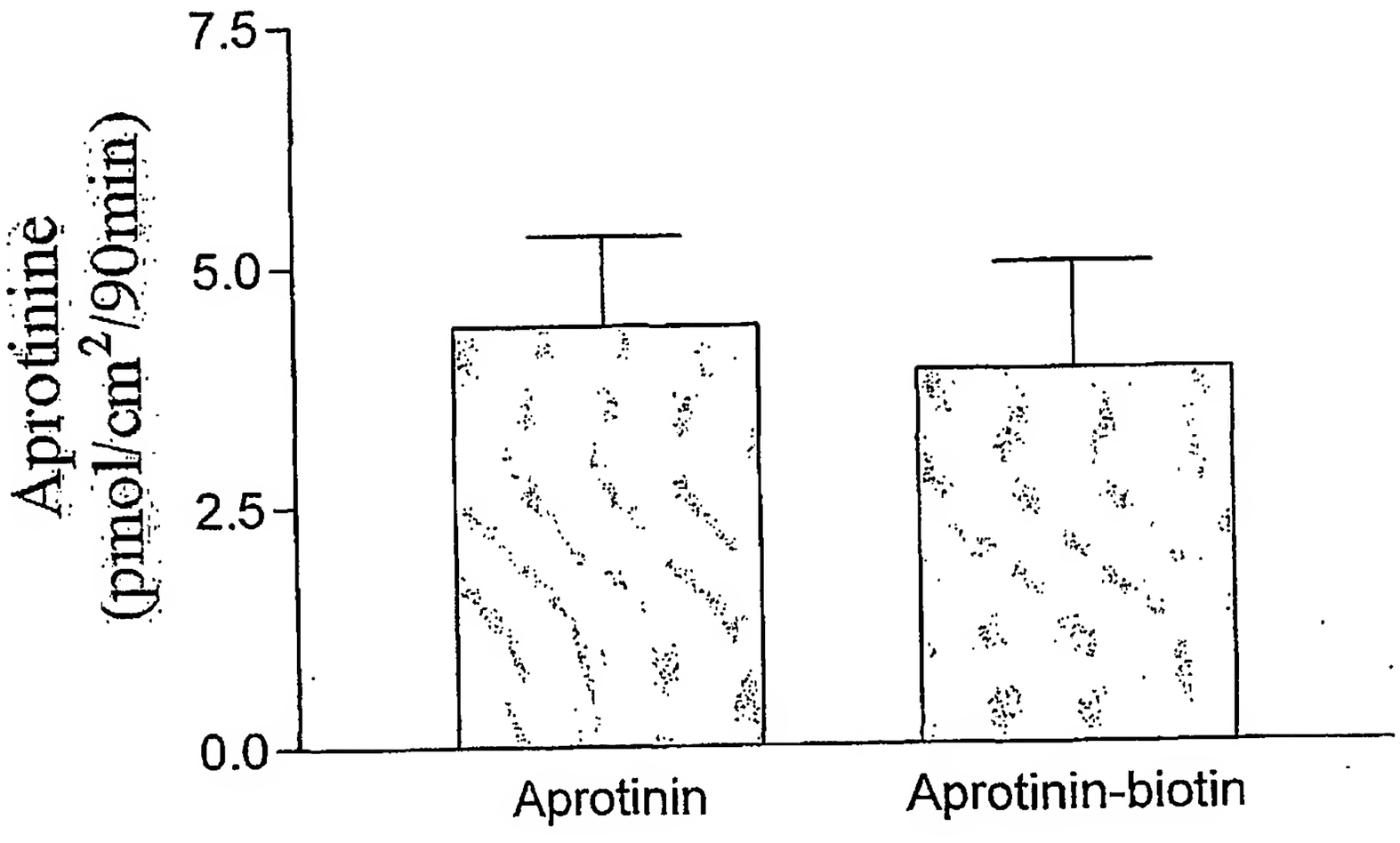

Aprotinine
(pmol/cm2/90min)
7.5
5.0
2.5
0.0
Aprotinin
Aprotinin-biotin

FIG. 10

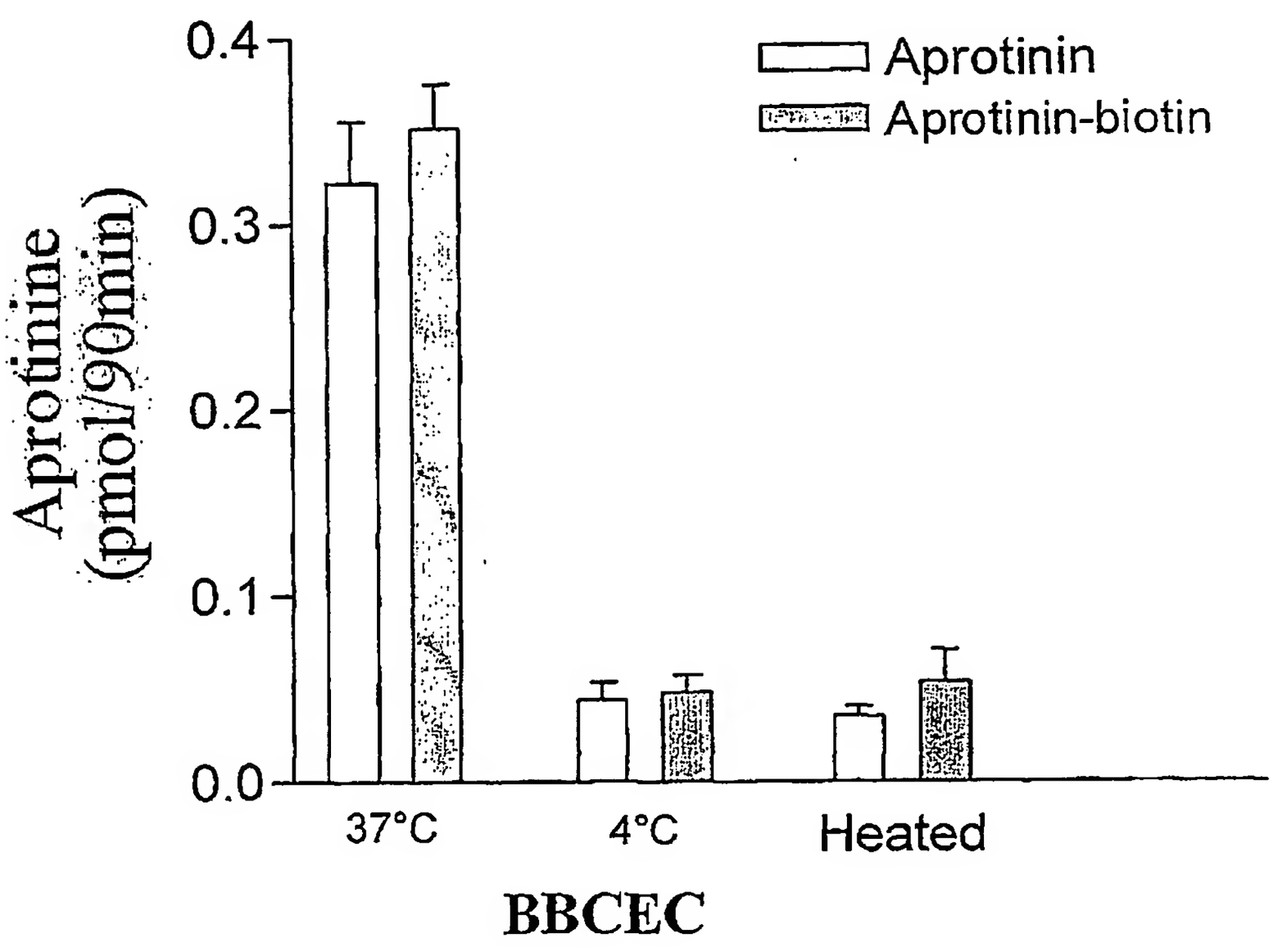

Aprotinin
Aprotinin-biotin
Aprotinine (pmol/90min)
0.4
0.3
0.2
0.1
0.0
37°C
4°C
Heated
BBCEC

FIG. 11

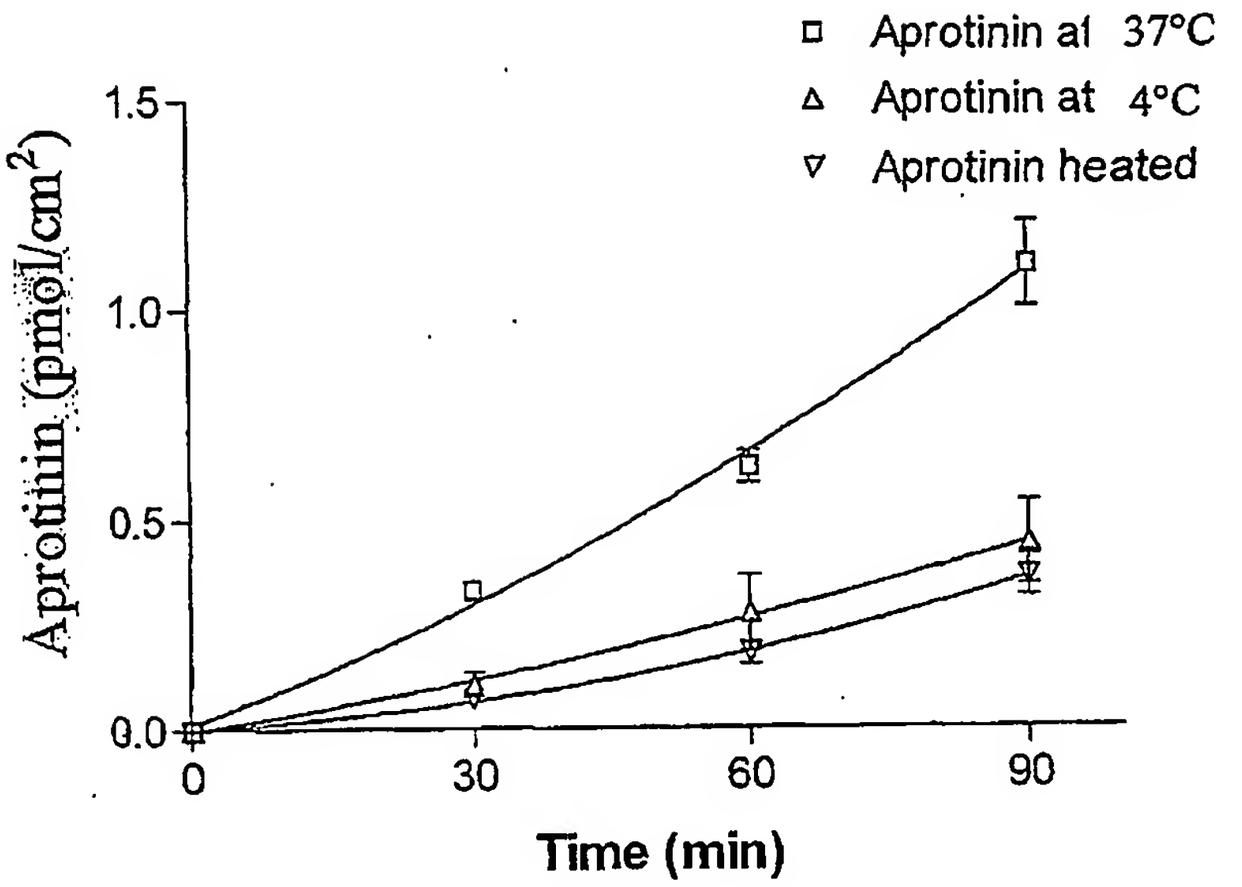


Fig. 12A

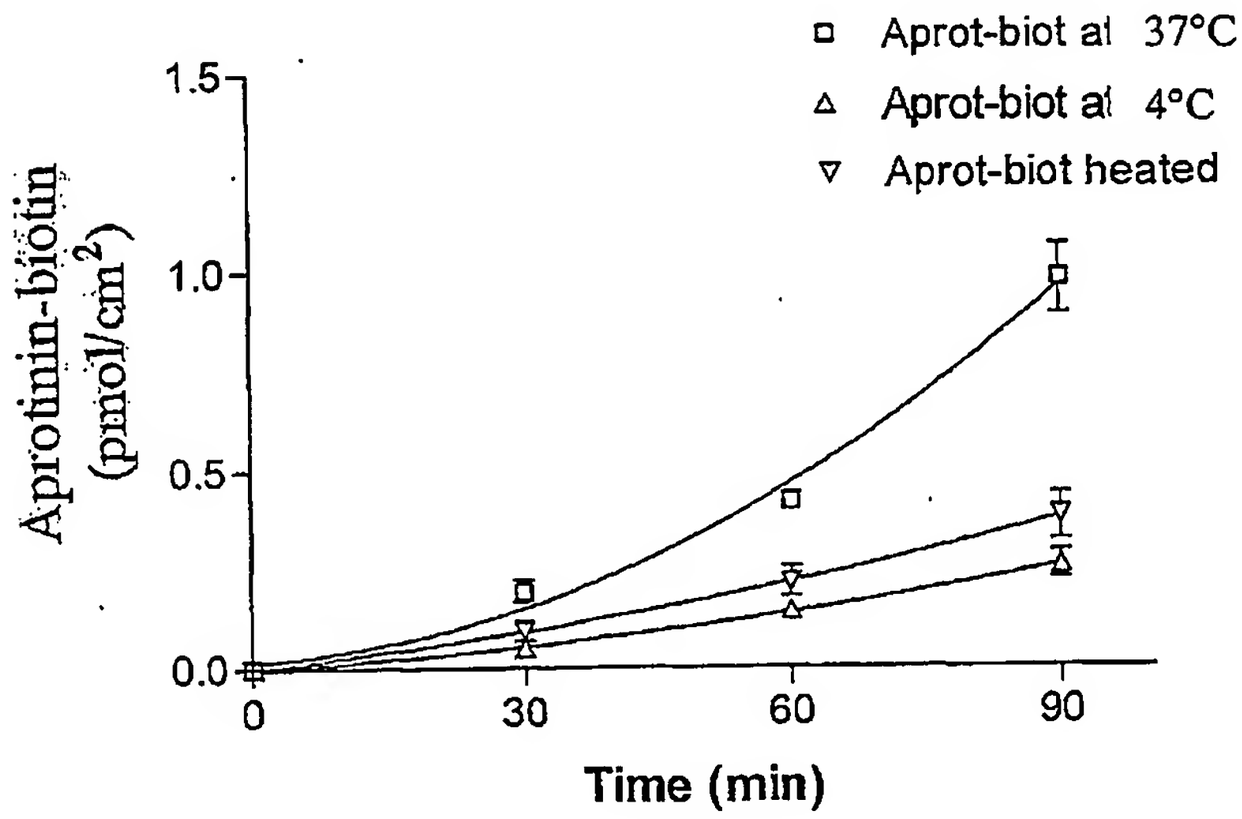


FIG. 12B

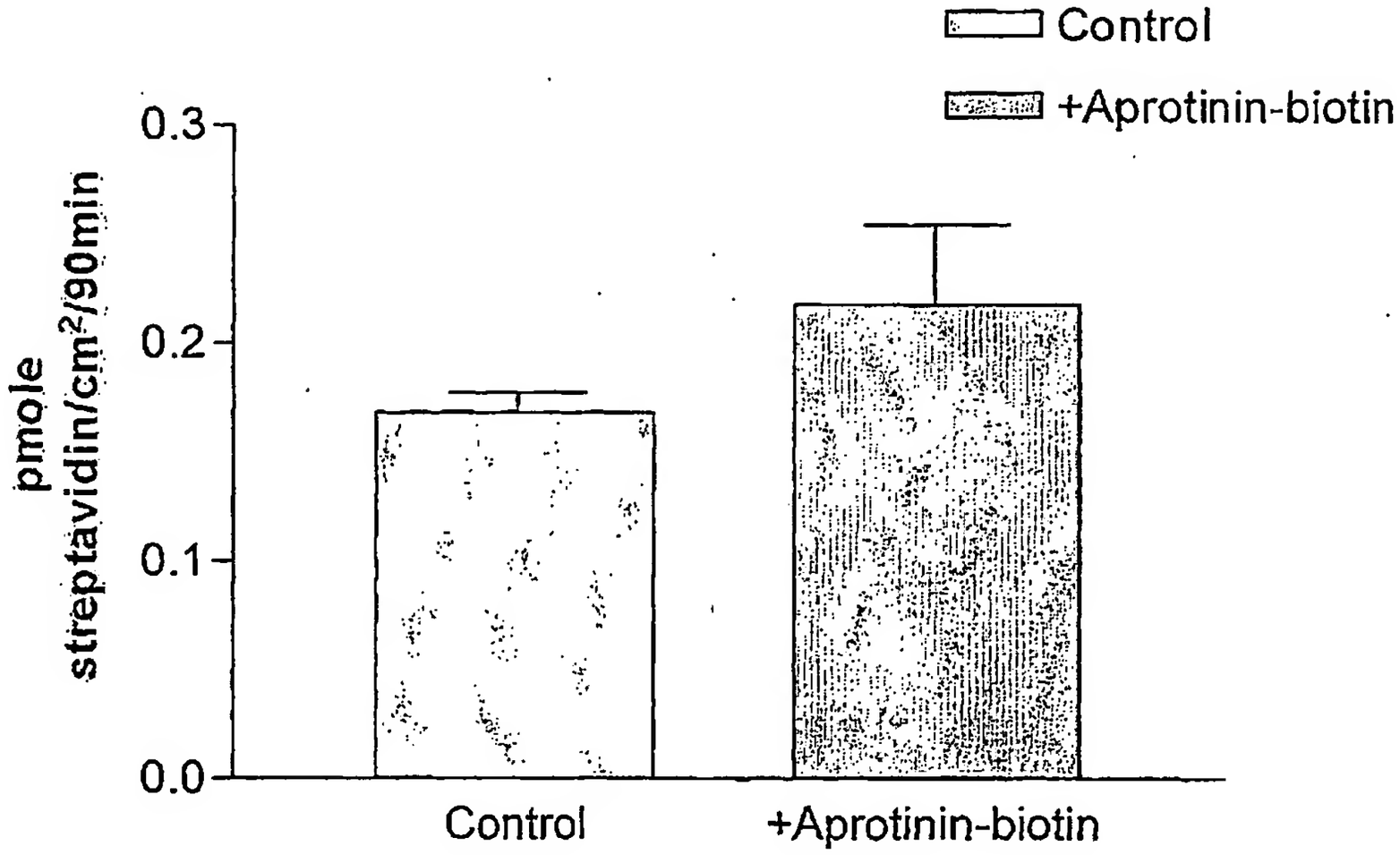

Control
+Aprotinin-biotin
pmole streptavidin/cm²/90min
0.3
0.2
0.1
0.0
Control
+Aprotinin-biotin

FIG. 13

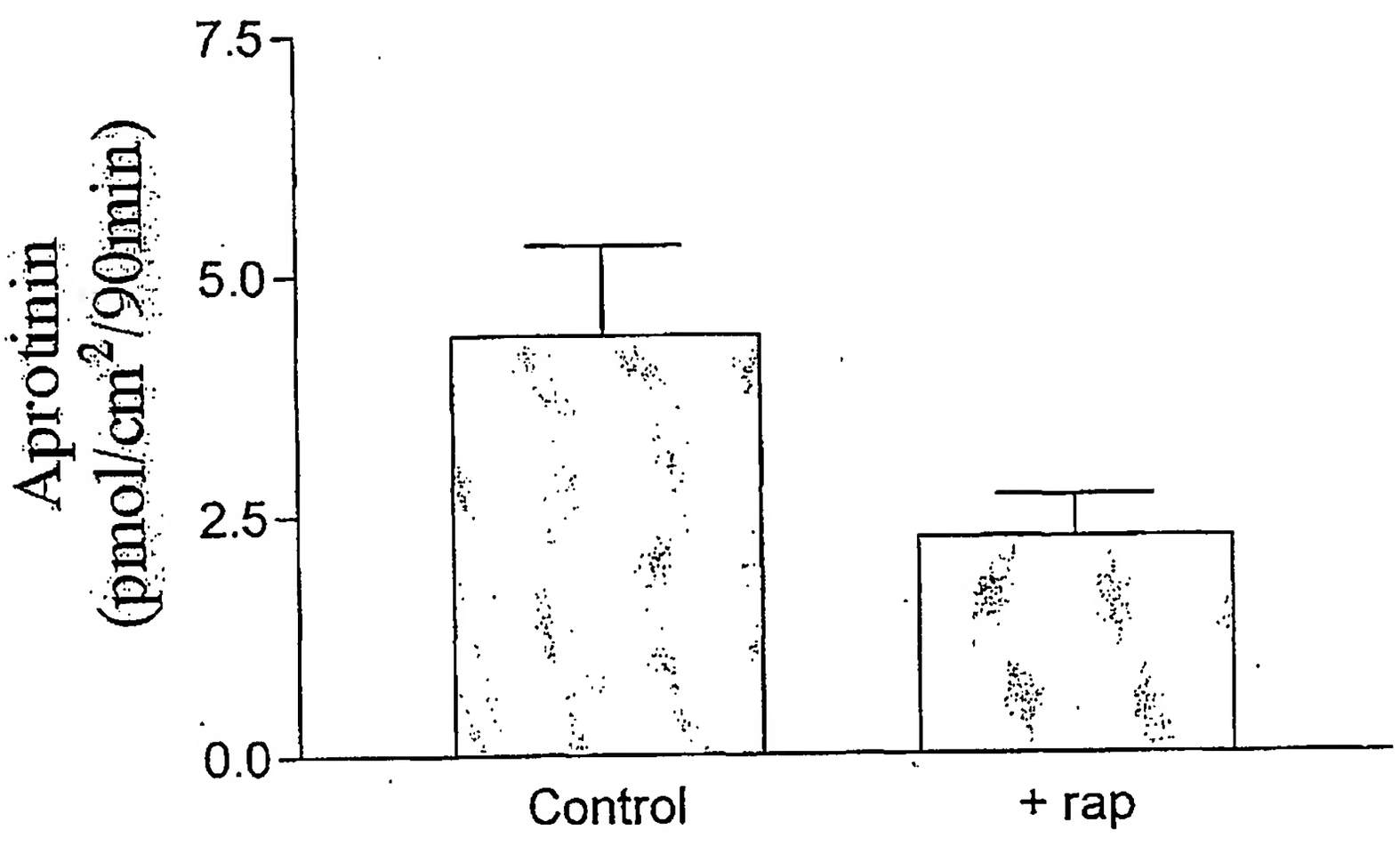
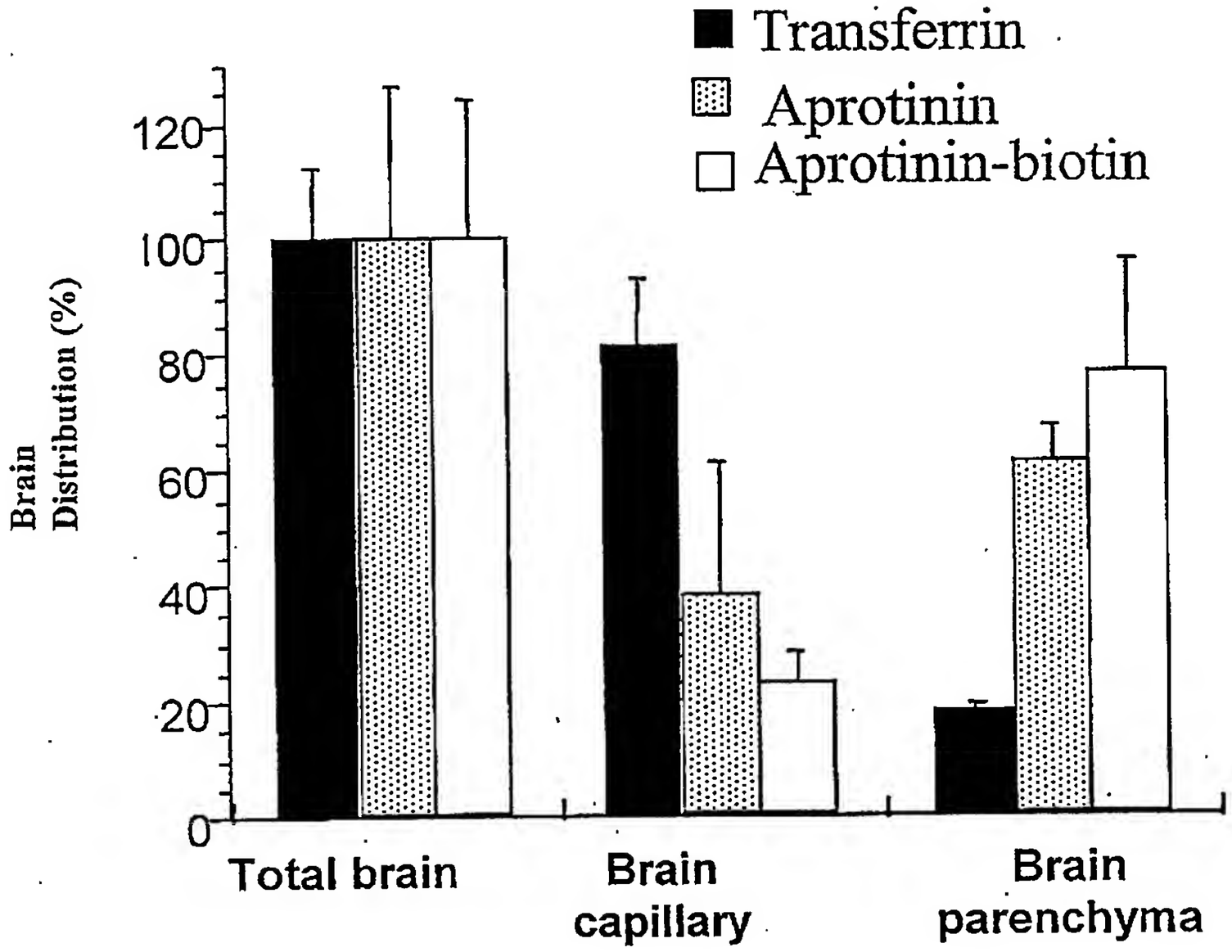


FIG. 14



V_D for aprotinin in brain parenchyma = 3 µl/100 g

FIG. 15

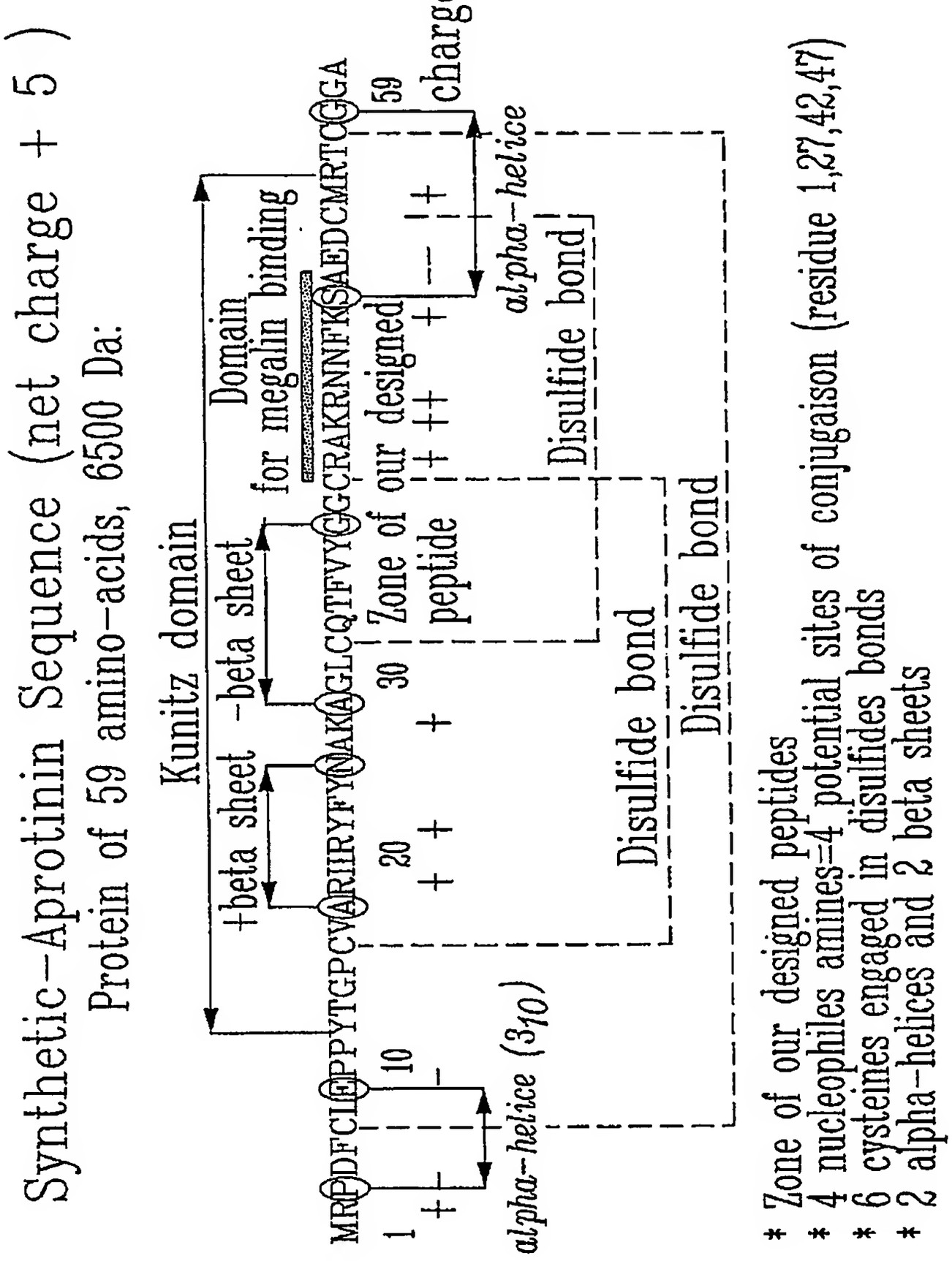
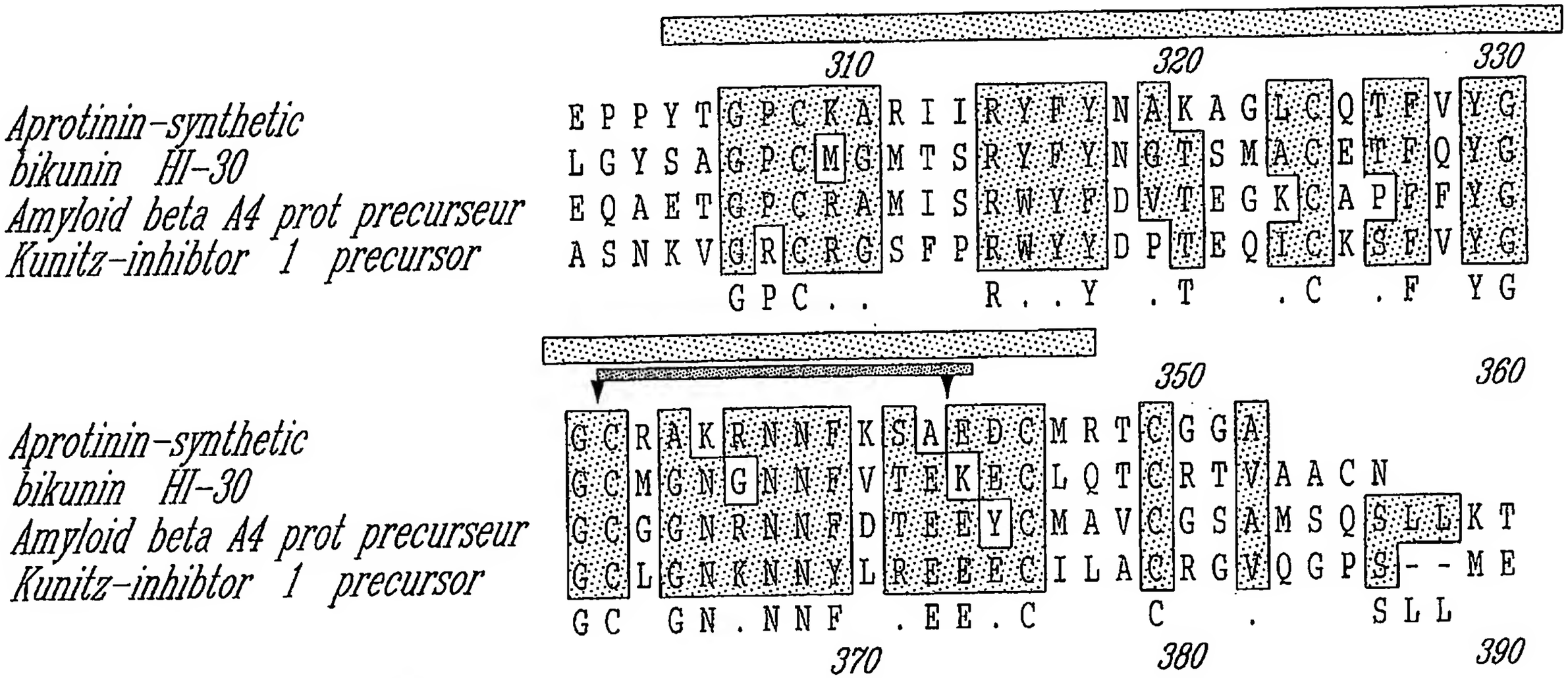

Synthetic–Aprotinin Sequence (net charge + 5)
Protein of 59 amino–acids, 6500 Da:
Kunitz domain
Domain
for megalin binding
+beta sheet
–beta sheet
Zone of our designed peptide
Disulfide bond
Disulfide bond
Disulfide bond
alpha–helice
alpha–helice (3₁₀)
charges
59
30
20
10
1
MRPDFCLEPPYTGPCVARIIRYFYNAKAGLCQTFVYGGCRAKRNNFKSAEDC
* Zone of our designed peptides
* 4 nucleophiles amines=4 potential sites of conjugaison (residue 1,27,42,47)
* 6 cysteines engaged in disulfides bonds
* 2 alpha–helices and 2 beta sheets

FIG. 16

FIG. 17

Alignement between aprotinin
and three human proteins with a similar domain



Kunitz domain

Mininmal sequence of aprotinin for its interaction with megalin

PCT/CA2004/000011

10/541304

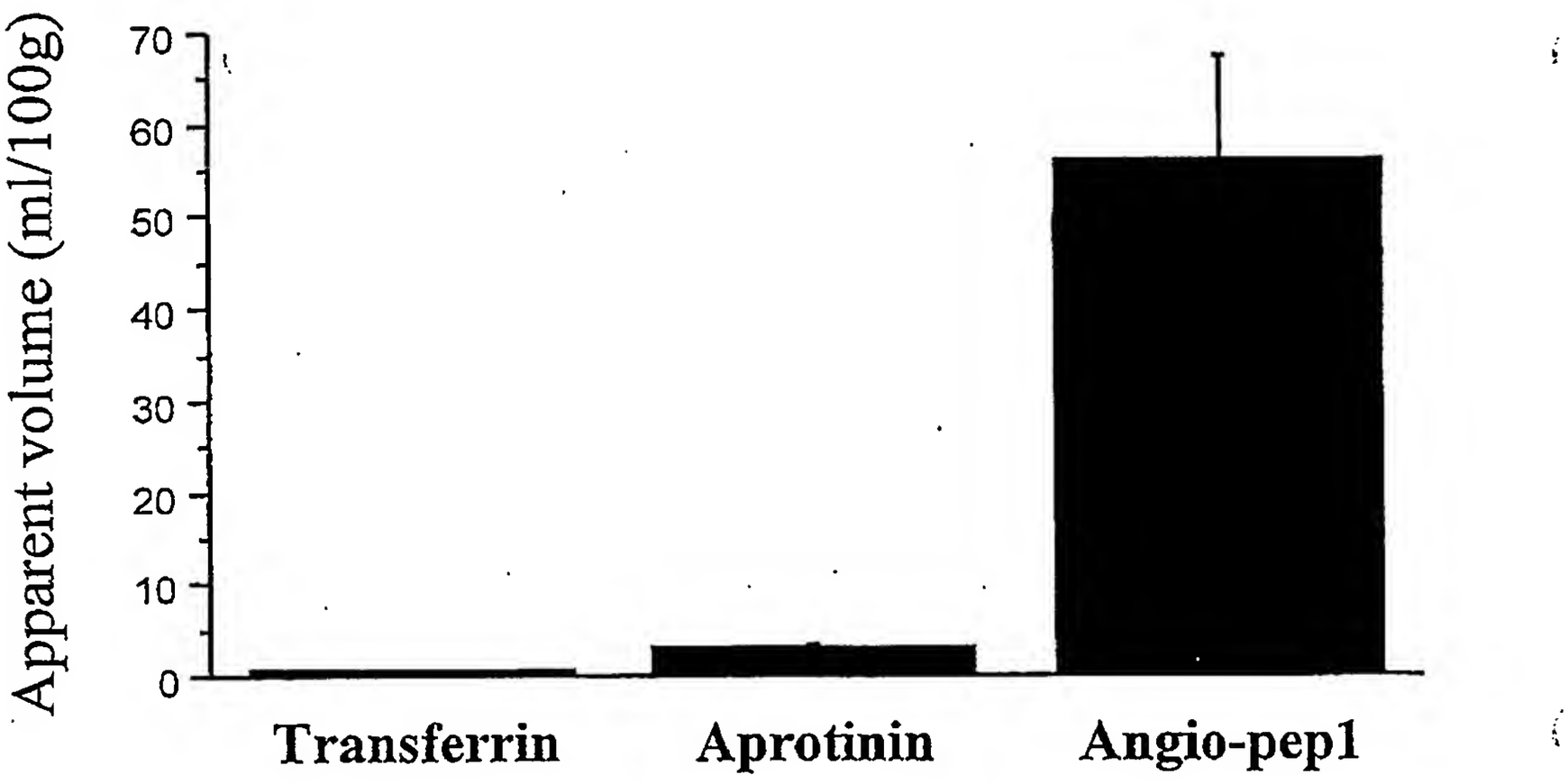

Apparent volume (ml/100g)
70
60
50
40
30
20
10
0
Transferrin
Aprotinin
Angio-pep1

FIG. 18

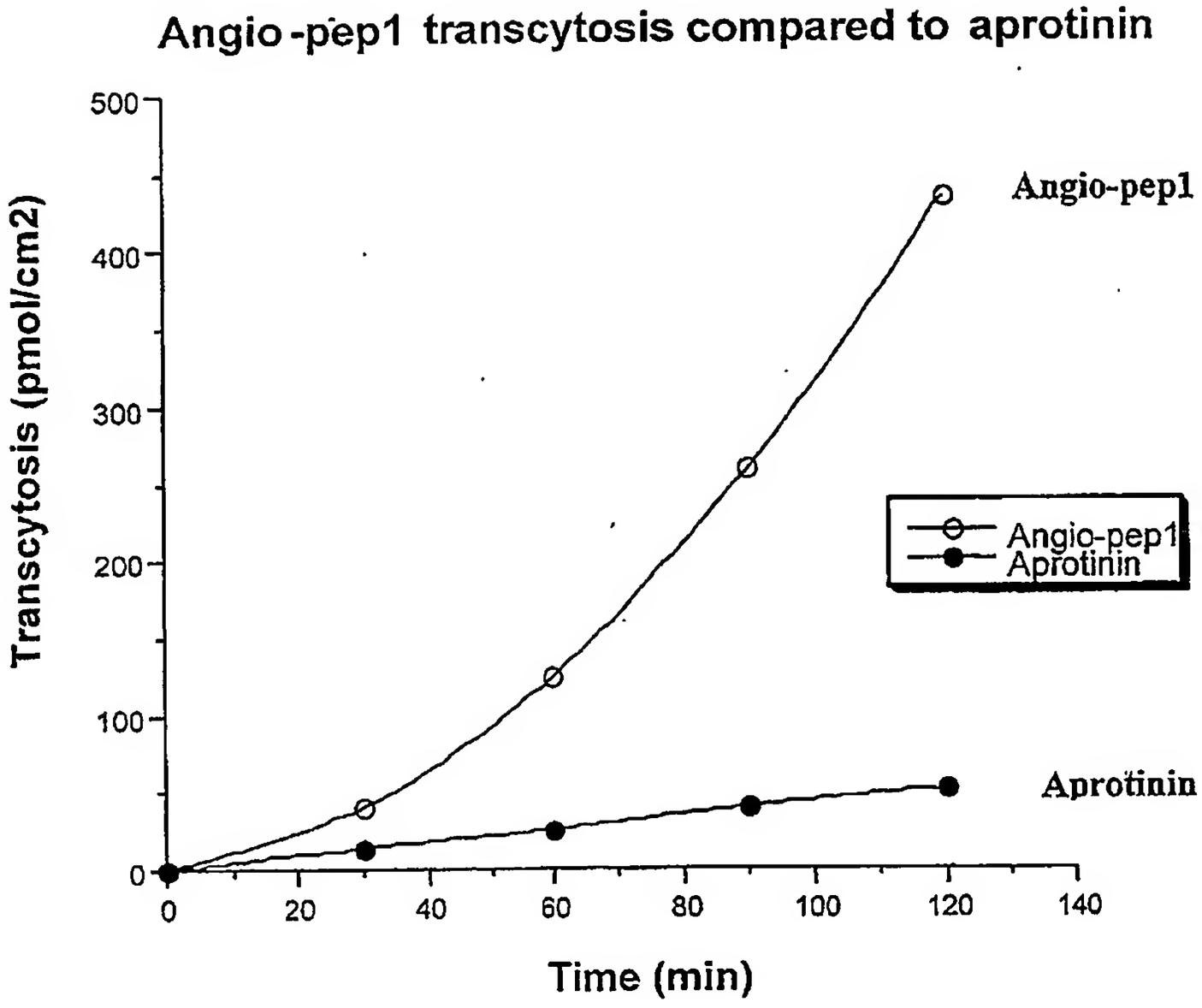

Angio-pep1 transcytosis compared to aprotinin
Transcytosis (pmol/cm2)
500
400
300
200
100
0
0
20
40
60
80
100
120
140
Time (min)
Angio-pep1
Aprotinin
Angio-pep1
Aprotinin

FIG. 19

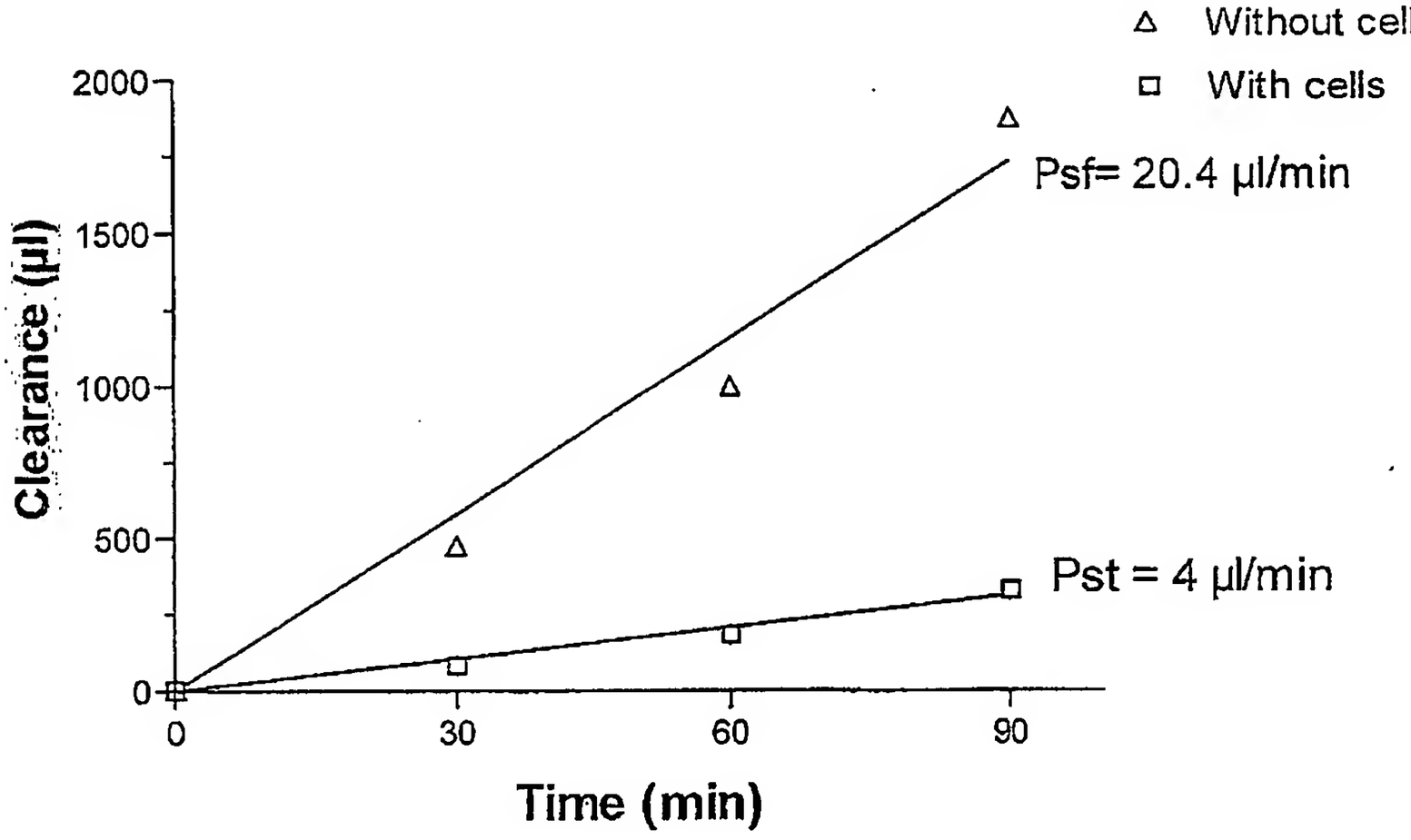

Without cells
With cells
Psf= 20.4 µl/min
Pst = 4 µl/min
Clearance (µl)
2000
1500
1000
500
0
0
30
60
90
Time (min)

FIG. 20